COMPARATIVE ANALYSIS OF GENETIC STRUCTURE AND DIVERSITY IN WILD LIMA BEAN POPULATIONS FROM THE CENTRAL VALLEY OF COSTA RICA, USING MICROSATELLITE AND ISOZYME MARKERS

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Introduction

A considerable amount of information has been accumulated on the genetic diversity and structure of the wild populations of Lima bean (*Phaseolus lunatus* L.) in the Central Valley of Costa Rica. Data on isozyme markers showed low interpopulation gene flow, a balance between among- and within- population variability, a predominantly autogamous breeding system and consequently a lack of heterozygosity in the sampled populations (Maquet *et al.*, 1996; Zoro, 1999; Maquet *et al.*, 2001). Field estimation of mating system parameters and analysis of factors influencing these parameters in the valley (such as pollen and seed dispersal, vegetative growth) confirmed also the mixed mating but predominantly selfing breeding system of wild Lima beans (Hardy *et al.*, 1997; Baudoin *et al.*, 1998).

Nevertheless, isozyme markers did not allow to analyze a high number of loci and revealed only a low level of allelic diversity in our case, which constitute limiting factors to study a key element in genetic structure of populations : gene flow at both short and long distance. Therefore, a study was initiated using higher polymorphic loci (i.e. microsatellites) and considering the maternal genotypes (leaves) rather than progenies (seeds). For this purpose, geographical coordinates of many wild populations in the Central Valley were encoded to obtain a distant matrix. In three representative regions of the valley (Escazú, Heredia and San Ramón), individuals of different populations were sampled in 2000 and 2001 from a central population and along a grid (each four meters in both directions) with the aim to measure gene flow using microsatellite markers.

Material and Methods

The first step was to develop a protocol of DNA extraction from leaves dried with silicagel and collected from populations in the Central Valley. The second step was to choose primers allowing DNA amplification and expressing polymorphism. For this purpose, we tested 73 primers isolated from *P. vulgaris* L. and provided by the Unit of Biotechnology at CIAT (Cali, Colombia). The third step was to determine the primer pairs likely to reveal polymorphism among individuals of wild populations directly collected along the grid established in the three regions of the Central Valley. The last step was to compare the parameters of population genetics obtained from the microsatellite and isozyme markers.

Results and Discussion

Using different protocols of DNA extraction, we retained with some adjustments the DNA extraction kit provided by Promega due to its lowest time consumption and efficiency for extracting DNA from dried old leaves. PCR reactions were carried out in 20µL mixtures containing 20 ng of genomic DNA template, 1 x reaction buffer, 0.1µM of each primer, 0.25 mM of each dNTPs, 1.5 U Taq DNA polymerase. After a thermocycle program, 5µL of PCR products were separated on 6% polyacrylamide gels in denaturing conditions for about 1h15 min at 1800 V and visualized using a silver staining procedure.

Out of 73 primer pairs isolated from *P. vulgaris*, 57 (78%) amplified in *P. lunatus*, with a good polymorphism obtained from 12 primer pairs : AG1, BM98, BM114, BM141, BM142, BM149, BM156, BM159, BM160, BM161, GAT554 and GAT591. Among the latter, four

primers AG1, BM160, BM161 and GAT554 were applied in a preliminary trial to amplify 191 individuals belonging to 10 populations distributed in Heredia. Sequencing gels were analyzed with the software GelCompar ver. 4.2. and the population genetics parameters were determined for the 191 individuals using the softwares FSTAT ver. 2.9.3.2. (Goudet, 1995) and Popgene ver. 3.2. (Yeh, Boylen, 1997). Table 1 show the first results obtained from Nei diversity indices (1973) and F-statistics (Weir, Cockerham, 1984).

Isozyme data were obtained from seeds collected during several years in 138 wild populations distributed in the whole valley and from the 13 following enzyme systems : ADH, DIA, END, EST, G6PDH, GDH, GPI, LAP, MDH, PGDH, PGM, SKDH and SOD. Number of individuals per population varies according to the period of collecting missions and the population size. Table 1 shows similar results between the two markers for F_{IT}, F_{IS} and F_{ST}. However, as expected by the studies of Ciofi *et al.* (1998), H_o, H_s, H_T and D_{ST} values are lower for isozymes than for microsatellites.

The same trend is observed in *Nm*, as expected by the study of Slatkin, Barton (1989).

Table 1. Genetic diversity indices and F-statistics in 10 wild Lima bean populations in the Central Valley of Costa Rica

parameter / marker	N_m [1]	H_o [2]	H_s [3]	H_T [4]	D_{ST} [5]	G_{ST} [6]	F_{IT} [7]	F
Microsatellites	0.17	0.031	0.215	0.489	0.273	0.560	0.924	0
Isozymes	0.04	0.012	0.060	0.186	0.126	0.680	0.929	0

[1] Nb of migrants according to Wright method, based on F_{ST}; [2] observed genetic diversity; [3] intrapopulation genetic diversity; [4] total genetic diversity; [5] interpopulation genetic diversity; [6] among population differenciation coefficient; [7] total consanguinity coefficient; [8] intrapopulation consanguinity coefficient; [9] fixation coefficient

Such preliminary results suggest a better estimation of gene flow and parameters of genetic diversity and structure with the use of microsatellites. Because the latter are more polymorphic and constitute co-dominant markers, a more precise measurement of gene flow using exclusion paternity analysis will be expected from all the leave samples taken in the three regions of the Central Valley. *We thank CIAT for providing the primers isolated from P. vulgaris.*

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